

**This item is the archived peer-reviewed author-version of:**

Immunocompetence and parasite infestation in a melanistic and normally-coloured population of the lacertid lizard, **\*\*Podarcis siculus\*\***

**Reference:**

Baeckens Simon, Van Damme Raoul.- Immunocompetence and parasite infestation in a melanistic and normally-coloured population of the lacertid lizard, **\*\*Podarcis siculus\*\***  
Amphibia-reptilia - ISSN 0173-5373 - 39:4(2018), p. 471-478  
Full text (Publisher's DOI): <https://doi.org/10.1163/15685381-20181008>  
To cite this reference: <https://hdl.handle.net/10067/1550590151162165141>

1           **Immunocompetence and parasite infestation in a melanistic and normally-**  
2                           **coloured population of the lacertid lizard, *Podarcis siculus***

3

4                           Simon Baeckens<sup>1,2,3</sup> and Raoul Van Damme<sup>1</sup>

5

6           <sup>1</sup>Laboratory of Functional Morphology, Department of Biology, University of  
7                           Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

8           <sup>2</sup>Museum of Comparative Zoology, Department of Organismic and Evolutionary  
9                           Biology, Harvard University, Cambridge, MA, USA

10

11                           <sup>3</sup> Corresponding author, email: [simon.baeckens@uantwerp.be](mailto:simon.baeckens@uantwerp.be)

12

13

14

15

16

Short note

17

18

19                           Short title: Immunity and melanism in *Podarcis siculus*

20

21

Word count abstract: 221

22

23

24

25 **Abstract.** Melanism is the occurrence of individuals that are darker in skin  
26 pigmentation than their conspecifics, which is a common colour polymorphism  
27 among vertebrates. Due to the pleiotropic effects of the POMC gene that is  
28 responsible for melanin-based colouration, dark pigmentation often co-varies with a  
29 range of other phenotypic traits. Still, not much is known on the link between  
30 melanin-based colouration and immunity in lizards. In this study, we examined and  
31 compared the immunocompetence and degree of ectoparasite infestation of  
32 *Podarcis siculus* lizards from a fully melanistic population on an islet in the  
33 Tyrrhenian Sea, with conspecifics from a 'normally'-coloured population on the  
34 mainland. Our findings show that both males and females from the melanistic  
35 population were less parasitized by ectoparasites and had a greater cellular immune  
36 response to a phytohemagglutinin injection than normally-coloured conspecifics.  
37 This outcome is in line with the "genetic link hypothesis", which predicts that  
38 melanistic individuals will be more resistant to parasites than non-melanistic  
39 individuals due to the pleiotropic POMC gene. In addition, we found correlative  
40 evidence for a link between ectoparasite load and PHA immune response, but this  
41 was only true for males from the normally-coloured population. Immunological data  
42 on additional melanistic and non-melanistic populations of *Podarcis siculus* in the  
43 Mediterranean basin would provide us better insight into patterns of co-variation  
44 between immunity and melanism in lizards.

45

46 *Keywords:* colour polymorphism; Licoso Islet; melanism; immunity; parasites; PHA;  
47 *Podarcis siculus*; POMC gene.

48

49

50

51

52

53

54

55

### Short Note

56 Melanism is the occurrence of individuals that are darker in skin pigmentation than  
57 their conspecifics (True 2003; Hoekstra 2006), which is a common colour  
58 polymorphism among ectotherms (Clusella Trullas et al. 2007), including lizards (e.g.  
59 Pérez-Mellado 1984; Zuffi 1986; Daniels et al. 2004; Trocsanyi and Korsos 2004;  
60 Korsos and Nagy 2006; San José et al. 2008; Parera et al. 2014). Natural selection  
61 may promote melanism directly through its effects on thermoregulation (e.g.  
62 Clusella-Trullas et al. 2008, 2009), crypsis (e.g. Kettlewell 1973; Endler 1984;  
63 Rosenblum 2006), aposematism (e.g. Turner 1977), sensitivity to UV radiation (e.g.  
64 Gunn 1998), disease resistance (e.g. Wilson et al. 2001), and mate selection (e.g.  
65 Wiernasz 1989). Alternatively, dark colouration may arise as a by-product of  
66 selection on other functions. Due to the pleiotropic effects of the gene responsible  
67 for melanin pigmentation in vertebrates (i.e. proopiomelanocortin [POMC] gene),  
68 melanin-based colouration often co-varies with a range of physiological and  
69 behavioural traits (reviewed in Ducrest et al. 2008). For instance, the POMC gene  
70 affects aggressiveness, food intake rate, energy expenditure, and sexual activity,  
71 besides pigmentation, so selection for any of these qualities would automatically  
72 result in darker colouration (Ducrest et al. 2008). An association between melanin  
73 pigmentation and immunity has also been documented, with dark individuals  
74 typically having a stronger anti-inflammatory response than lighter individuals. For  
75 instance, Roulin et al. (2000, 2001) showed a relationship between the degree of  
76 pigmentation and immune response and parasite resistance in barn owls (*Tyto alba*).  
77 In tawny owls (*Strix aluco*) and feral pigeons (*Columba livia*) dark individuals can  
78 maintain stronger levels of antibody production over longer periods of time

79 compared to pale conspecifics (Gasparini et al. 2009; Jacquin et al. 2011). While the  
80 link between melanin-based colouration and immunity and parasite load is well  
81 documented in birds and mammals (Ducrest et al. 2008), it is less so in lizards  
82 (Vroonen et al. 2013; Seddon and Hews 2016).

83 In this study, we examined and compared the immunocompetence and  
84 degree of ectoparasite infestation of *Podarcis siculus* lizards from a melanistic  
85 population with their conspecifics from a 'normally'-coloured population. The  
86 melanistic lizards of study originated from a population found on a small island in the  
87 Tyrrhenian Sea near the Italian coast, called Licosa Islet. Individuals of both sexes are  
88 melanistic and no normally-coloured lizard has ever been observed on the island  
89 (Raia et al. 2010; Monti et al. 2013; personal observation). Conspecifics on the  
90 mainland have the green-brown dorsal colours typical of the species. Previous  
91 research on this particular study system already showed that lizards from the  
92 melanistic population of Licosa Islet exhibit increased activity of the POMC gene and  
93 its posttranslational products (Raia et al. 2010; Monti et al. 2013; Trapanese et al.  
94 2017), and provided some support for the pleiotropic POMC effect. Raia et al. (2010)  
95 observed more aggressive interactions among melanistic lizards of Licosa Islet than  
96 among normally-coloured conspecifics on the mainland, and Monti et al. (2013)  
97 established a difference in reproductive output, with melanistic females having a  
98 larger clutch mass than normally-coloured females on the mainland. The latter  
99 researchers also assessed the lizards' ectoparasite load and found that male lizards  
100 from Licosa Islet were more infested than male conspecifics from the mainland.  
101 However, these findings are based on a relative low sample size of solely male  
102 individuals ( $N = 30$ ). We gathered data on cell-mediated immunity and ectoparasite

103 infestation of a large number of individuals ( $N = 106$ ) from both sexes and from both  
104 populations. We hypothesize that due to the pleiotropic effects of the POMC gene,  
105 both male and female lizards from the melanistic population of Licosia will be  
106 equipped with a greater immune response and will be less parasitized than their  
107 normally-coloured conspecifics on the mainland.

108         In September 2012, we caught 57 adult *Podarcis siculus* lizards (27 males, 30  
109 females) of the melanistic population on the island of Licosia (40°15'05.0"N,  
110 14°54'00.8"E), and 49 normally-coloured on the mainland (23 males, 26 females)  
111 near the village Castellabata, in Italy (40°14'59.8"N, 14°54'37.3"E) (Fig. 1). Lizards  
112 were captured by noose and transported individually in cotton bags to the field lab  
113 on the mainland. In the lab, the degree of ectoparasite infestation of each lizard was  
114 assessed by counting the number of ticks (Ixodidae) and mites (Trombiculidae) on  
115 the lizard' skin surface. The cloth bags were also checked for possible ectoparasites,  
116 which the lizard may have lost during transport. Mite count and tick count were later  
117 pooled into one measure ('ectoparasite count') and used in further statistical  
118 analyses. Lizards were, thereafter, kept per population (sexes separately) in large  
119 meshed cages (1.2 x 0.5 x 0.7 m). Water and food (wax moth larvae, *Achroia grisella*)  
120 were provided *ad libitum*. To estimate the immunocompetence of each lizard, we  
121 quantified its delayed cutaneous hypersensitivity response (Belluire et al. 2004;  
122 Oppliger et al. 2004) by injecting one foot of every individual with a 20  $\mu$ L solution  
123 containing 30 mg of phytohaemagglutinin (PHA; Sigma–Aldrich, L-8754) in 5 mL  
124 phosphate buffered saline (PBS). PHA influences a variety of cell types and, therefore  
125 the response to PHA injection is complex, but can serve as an index for heightened  
126 immune cell activity (Kennedy and Nager 2006; Martin et al. 2006). Thickness of the

127 foot was measured before injection and 24 hours later using a digital calliper  
128 (Mitutuyo; accuracy: 0.01 mm). The other foot was treated in the same way, but  
129 injected with 20  $\mu$ L of PBS serving as a control. The immune response was calculated  
130 as the change in thickness of the PHA injected foot minus the change in the control  
131 foot. Larger localized swelling indicated an increased immune activity at the cellular  
132 level. Also the snout-vent length (SVL) and body mass (Balance EC 100; accuracy:  
133 0.01 g) of each lizard was measured. Body condition was estimated as the residuals  
134 of a linear regression of body mass on SVL. Prior to any statistical analyses in R,  
135 variables were  $\log_{10}$  (SVL, mass) or square root (ectoparasite count) transformed to  
136 meet assumptions of normality (Shapiro-Wilks test:  $W \geq 0.95$ ). Linear regressions  
137 were used to explore patterns of variation in ectoparasite load, immune response,  
138 SVL, and body condition between sexes and populations.

139         The proportion of lizards infested with ectoparasites was greater in the  
140 normally-coloured mainland population (100%) than the melanistic island population  
141 (81%) (Z-score = 3.25,  $P = 0.001$ ). Also the degree of infestation was significantly  
142 higher in normally-coloured lizards than in melanistic lizards ( $F_{1,103} = 115.82$ ,  $P <$   
143  $0.001$ ; Fig. 2a). In the normally-coloured population, but not in the melanistic  
144 population, males were more severely infested than females (population \* sex  
145 interaction;  $F_{1,102} = 9.66$ ,  $P = 0.002$ ; Fig. 2a). Further, melanistic lizards exhibited a  
146 significantly greater PHA immune response than normally-coloured lizards ( $F_{1,103} =$   
147  $19.93$ ,  $P < 0.001$ , Fig. 2b). There were no sex differences in immune response ( $F_{1,103} =$   
148  $0.74$ ,  $P = 0.392$ , Fig. 2b). In normally-coloured males, ectoparasite load was positively  
149 correlated with PHA immune response ( $F_{1,17}=4.93$ ,  $P = 0.040$ ). In the other groups,  
150 the relationship was not significant (normally-coloured females:  $P = 0.221$ ; melanistic

151 males:  $P = 0.927$ ; melanistic females:  $P = 0.416$ ). Lizards on the island of Licosa did  
152 not differ in SVL from their conspecifics on the mainland ( $F_{1,103} = 0.48$ ,  $P = 0.482$ ).  
153 While males were larger than females in both populations ( $F_{1,103}=31.82$ ,  $P < 0.001$ ),  
154 the degree of sexual size dimorphism was higher in the normally-coloured  
155 population (population \* sex interaction;  $F_{1,102} = 4.78$ ,  $P = 0.031$ ). On average,  
156 normally-coloured lizards had a higher body condition than melanistic lizards ( $F_{1,103} =$   
157  $34.79$ ,  $P < 0.001$ ). Body condition was higher in males than females, and this sex  
158 difference was most pronounced in the normally-coloured population (population \*  
159 sex interaction;  $F_{1,102} = 5.75$ ,  $P = 0.018$ ). For neither sex nor population, we found a  
160 significant relationship between body condition and ectoparasite load (all  $F < 1.4$ ,  $P >$   
161  $0.24$ ), and immune response (all  $F < 2.4$ ,  $P > 0.13$ ).

162 Our findings show that *Podarcis siculus* lizards from the melanistic population  
163 of Licosa Islet were less parasitized by ectoparasites and had a greater cellular  
164 immune response to PHA injection than normally-coloured conspecifics on the  
165 nearby mainland, and this was true for both males and females. This outcome is in  
166 line with the “genetic link hypothesis” (Jacquin et al. 2011), which predicts that  
167 melanistic individuals will be more resistant to parasites than non-melanistic  
168 individuals due to the pleiotropic effects of the POMC gene (Ducrest et al. 2008).  
169 Similar findings have been documented in birds and mammals (Roulin and Ducrest  
170 2011), although the few studies that have focused on lizards show equivocal results  
171 on the link between melanism and immunity. In *Zootoca vivipara*, males (but not  
172 females) with a higher degree of ventral melanin pigmentation mounted a stronger  
173 PHA-induced immune response than males of the same population that have less  
174 pigmentation (Vroonen et al. 2013). No significant relationship was found between

175 degree of pigmentation and tick infestation (Vroonen et al. 2013). Seddon and Hews  
176 (2016) studied two populations of *Sceloporus occidentalis* lizards, which differed  
177 substantially in their degree of melanisation. Male lizards originating from the  
178 darkly-coloured population were found to be more infested with mites than males  
179 from the pale-coloured population. However, within population variation in the  
180 degree of melanin pigmentation was not significantly related with mite load (Seddon  
181 and Hews 2016). Part of these discordant findings among studies might be attributed  
182 to the large variation in melanisation among the lizards of study and/or the timing of  
183 sampling. Firstly, our study compares two populations that drastically differ in  
184 melanisation (complete melanistic vs. complete non-melanistic), while previous  
185 studies compared lizards that only vary slightly in their degree of pigmentation (such  
186 as Vroonen et al. 2013, and Seddon and Hews 2016). It would be interestingly to  
187 gather immunological data on several full melanistic and non-melanistic populations  
188 of *Podarcis siculus* in the Mediterranean basin in order to gain a better insight into  
189 patterns of co-variation between immunocompetence and melanism in lizards.  
190 Secondly, while the aforementioned studies by Vroonen et al. (2013) and Seddon  
191 and Hews (2016) were conducted early in reproductive season (May-June), we  
192 surveyed our study site near the end of the season (September). Temporal variation  
193 in parasite abundance (which can moreover differ among localities; Brito et al.  
194 2014), but also lizard immune function (Huyghe et al. 2009), might obscure clear  
195 patterns of covariance between melanisation and immunity. Clearly, future studies  
196 should invest in collecting information at multiple times throughout the reproductive  
197 season.

198

199           Why would melanism have evolved in lizards on Licoso Islet? Atypical  
200 pigmentation on islands has been explained as an adaptive response to thermal  
201 conditions (Ortega and Pérez-Mellado 2016), to differential predation pressures  
202 (Cooper and Pérez-Mellado 2012), or a combination of both (Bittner and King 2003;  
203 Tanaka 2007). In this scenario, the thermal benefits of a dark pigmentation (Clusella-  
204 Trullas et al. 2007, 2008, 2009) combined with the loss of the need for camouflage  
205 (Gibson 1978; Vervust et al. 2007) may have driven the evolution of melanism on  
206 Licoso Islet, and the accompanied high immunocompetence would have then arose  
207 as a by-product ('spandrel') due to the pleiotropic POMC gene. While the "genetic  
208 link hypothesis" is one explanation on why we found differences in ectoparasite load  
209 and immunocompetence between lizards from Licoso Islet and lizards from the  
210 mainland, there are, at least, four alternative explanations. Firstly, while our findings  
211 indicate that melanistic lizards carry a lower number of parasites because they have  
212 a better immunocompetence than normally-coloured lizards, we can not rule out  
213 that the observed differences in parasite infestation between populations might be  
214 the result of differences in parasite abundance. There are cases in birds and  
215 mammals, where interpopulational variation in parasite abundance has been  
216 reported as an important driver of variation in parasite infestation (Poulin 2006;  
217 Santiago-Alarcon et al. 2008). Since parasite communities on islands often occur in  
218 low densities (Nieberding et al. 2006), the low degree of parasite infestation in  
219 Licoso lizards might, therefore, be the result of overall low parasite abundance on  
220 Licoso Islet. Moreover, we were unable to find correlative evidence for a direct link  
221 between parasite load and immunocompetence in our lizards of study, with the  
222 exception of mainland males where we established a significant positive correlation.

223 The direction of correlation was, however, not as expected, as we predicted that the  
224 immune response to PHA-injection would be negatively (not positively) correlated  
225 with the intensity of parasite infection, e.g. as recorded in *Podarcis muralis* (Amo et  
226 al. 2005). However, a positive correlation has also been observed, i.e. in males of the  
227 lizard *Podarcis melisellensis* (Huyghe et al. 2009). The latter researchers argue that  
228 this result could be a priming effect, in which lizards with high loads of ectoparasites  
229 may already be primed for immunological responses (PHA swelling) because their  
230 immune system is not constantly challenged. Those with decreased ectoparasite  
231 loads may then exhibit a lowered swelling response, as their immune systems have  
232 not been primed in the same way. It would be interesting to evaluate the link  
233 between endoparasite load (e.g. haemogregarines, coccidian, nematodes) and PHA  
234 swelling response, and whether it varies (or not) with ectoparasite load. Secondly,  
235 differences in immunocompetence between populations could also be ascribed to  
236 divergent levels of corticosterone due to differences in stress. Elevated levels of  
237 corticosterone as a result of stress can suppress immune activity (Berger et al. 2005)  
238 and increase parasite infection (Oppliger et al. 1998). One can imagine that lizards on  
239 the mainland are exposed to higher levels of stress than island lizards, due to high  
240 predation pressure (from both natural and domestic predators) on the mainland in  
241 comparison to the island (e.g. Pérez-Mellado et al. 1997; Vervust et al. 2007), or due  
242 to the high level of interspecific competition for resources on the mainland relative  
243 to the island (Case and Bolger 1991). This hypothesis could be tested by comparing  
244 corticosterone levels between lizards of both populations (Huyghe et al. 2009).  
245 Thirdly, the evolution of melanism on Licoso Islet might be a by-product of selection  
246 for immunocompetence. If parasite resistance is more important (relative to other

247 functions) on Licosia Islet than on the mainland, insular melanism may arise as a  
248 secondary result. However, this scenario is highly unlikely, as parasite abundance is  
249 generally lower on islands than on the mainland (Nieberding et al. 2006). Fourthly,  
250 diversity in pigmentation and/or immunity can also occur through factors such as  
251 genetic drift, founder effects, and inbreeding (e.g. Runemark et al. 2010), factors  
252 that are typically more common in island than mainland populations (Nevo 1978).

253 In conclusion, the findings of this study show differences in immunity and  
254 parasite infestation between lizards from a melanistic and non-melanistic  
255 population. While the “genetic link hypothesis” is one potential explanation for this  
256 outcome, additional research is necessary to determine the true underlying  
257 mechanism. We hope that this short note encourages further research on the link  
258 between melanin-based colouration and immunity in lizards.

259

## 260 **Acknowledgments**

261 We thank Domenico Fulgione, Jessica Vroonen, and Billy Dries for assistance in the  
262 field, and two anonymous reviewers for their constructive feedback on a previous  
263 draft of the manuscript. All experiments described were performed in accordance  
264 with local and national guidelines governing animal experiments, and under permits  
265 issued by the University of Naples Federico II. Simon Baeckens is a postdoctoral  
266 fellow supported by the Belgian American Education Foundation (B.A.E.F.). This  
267 study is part of a larger project supported by the FWO – Flanders (project number  
268 G.0092.11N).

269

## 270 **References**

271 Amo, L., Fargallo, J.A, Martínez-Padilla, J., Millán, J., López, P., Martín, J. (2005):  
272 Prevalence and intensity of blood and intestinal parasites in a field population  
273 of a Mediterranean lizard, *Lacerta lepida*. Parasitol. Res. **96**: 413–7.

274 Belliure, J., Smith, L., Sorci, G. (2004): Effect of testosterone on T cell-mediated  
275 immunity in two species of Mediterranean lacertid lizards. J. Exp. Zool. A.  
276 Comp. Exp. Biol. **301**: 411–418.

277 Berger, S., Martin I.I., L.B., Wikelski, M., Romero, L.M., Kalko, E.K.V., Vitousek, M.N.,  
278 Rödl, T. (2005): Corticosterone suppresses immune activity in territorial  
279 Galapagos marine iguanas during reproduction. Horm. Behav. **47**: 419–429.

280 Brito, S.V., Ferreira, F.S., Ribeiro, S.C., Anjos, L.A., Almeida, W.O., Mesquita, D.O.,  
281 Vasconcellos A. (2014): Spatial-temporal variation of parasites in  
282 *Cnemidophorus ocellifer* (Teiidae) and *Tropidurus hispidus* and *Tropidurus*  
283 *semitaeniatus* (Tropiduridae) from Caatinga areas in northeastern Brazil.  
284 Parasitol. Res. **113**: 1163–1169.

285 Case, T.J. and Bolger, D.T. (1991): The role of interspecific competition in the  
286 biogeography of island lizards. Trends Ecol. Evol. **6**: 135-139.

287 Clusella-Trullas, S., van Wyk, J.H., Spotila, J.R. (2007): Thermal melanism in  
288 ectotherms. J. Therm. Biol. **32**: 235–245.

289 Clusella-Trullas, S., Terblanche, J.S., Blackburn, T.M., Chown, S.L. (2008): Testing the  
290 thermal melanism hypothesis: a macrophysiological approach. Funct. Ecol. **22**:  
291 232–238.

292 Clusella-Trullas, S., Van Wyk, J.H., Spotila, J.R. (2009): Thermal benefits of melanism  
293 in cordylid lizards: a theoretical and field test. Ecology **90**: 2297–2312.

294 Cooper W.E., Pérez-Mellado, V. (2012): Historical influence of predation pressure on  
295 escape by Podarcis lizards in the Balearic Islands. Biol. J. Linn. Soc. **107**: 254–  
296 268.

297 Daniels, S.R., Mouton Le F.N., Toit, D.A. (2004): Molecular data suggest that  
298 melanistic ectotherms at the south-western tip of Africa are the products of  
299 Miocene climatic events: evidence from cordylid lizards. J. Zool. **263**: 373–383.

300 Ducrest, A.L., Keller, L., Roulin, A. (2008): Pleiotropy in the melanocortin system,  
301 coloration and behavioural syndromes. Trends Ecol. Evol. **23**: 502–510.

302 Endler, J. (1984): Progressive background matching in moths, and a quantitative  
303 measure of crypsis. Biol. J. Linn. Soc. **2**: 187–231.

304 Gasparini, J., Bize, P., Piau, R., Wakamatsu, K., Blount, J.D., Ducrest, A.L., Roulin, A.  
305 (2009): Strength and cost of an induced immune response are associated with a  
306 heritable melanin-based colour trait in female tawny owls. J. Anim. Ecol. **78**:  
307 608–16.

308 Gibson, A.R. (1978): The ecological significance of a colour polymorphism in the  
309 common garter snake, *Thamnophis sirtalis* (L.) Unpublished PhD Dissertation.  
310 Toronto: University of Toronto.

311 Gunn, A. (1998): The determination of larval phase coloration in the African  
312 armyworm, *Spodoptera exempta* and its consequences for thermoregulation  
313 and protection from UV light. Entomol. Exp. Appl. **86**: 125–133.

314 Hoekstra, H.E. (2006): Genetics, development and evolution of adaptive  
315 pigmentation in vertebrates. Heredity **97**: 222–234

316 Huyghe, K., Van Oystaeyen, A., Pasmans, F., Tadić, Z., Vanhooydonck, B. & Van  
317 Damme, R. (2010): Seasonal changes in parasite load and a cellular immune  
318 response in a colour polymorphic lizard. *Oecologia* **163**: 867–74.

319 Huyghe, K., Husak, J.F., Herrel, A., Tadić, Z., Moore, I.T., Van Damme, R.,  
320 Vanhooydonck, B. (2009): Relationships between hormones, physiological  
321 performance and immunocompetence in a color-polymorphic lizard species,  
322 *Podarcis melisellensis*. *Horm. Behav.* **55**: 488–494.

323 Jacquin, L., Lenouvel, P., Haussy, C., Ducatez, S., Gasparini, J. (2011): Melanin-based  
324 coloration is related to parasite intensity and cellular immune response in an  
325 urban free living bird : the feral pigeon *Columba livia*. *J. Avian Biol.* **42**: 11–15.

326 Janzen, D.H. (1973): Sweep samples of tropical foliage insects: Effects of seasons,  
327 vegetation types, elevation, time of day and insularity. *Ecology* **54**: 687–708.

328 Kennedy, M.W., Nager, R.G. (2006): The perils and prospects of using  
329 phytohaemagglutinin in evolutionary ecology. *Trends Ecol. Evol.* **21**: 653–655.

330 Kettlewell, H.B.D. (1973): *The Evolution of Melanism: The Study of a Recurring*  
331 *Necessity, With Special Reference to Industrial Melanism in the Lepidoptera.*  
332 *Clarendon Press, Oxford.*

333 Korson, Z., Nagy, Z.T. (2006): Short report on a completely melanistic specimen of  
334 the East European green lizard *Lacerta viridis* (Laurenti, 1768), in Hungary.  
335 *Eidechse* **17**:42–46.

336 Martin, L.B., Han, P., Lewittes, J., Kuhlman, J.R., Klasing, K.C., Wikelski, M. (2006):  
337 Phytohemagglutinin-induced skin swelling in birds: histological support for a  
338 classic immunoeological technique. *Funct. Ecol.* **20**: 290–299.

339 Monti, D.M., Raia, P., Vroonen, J., Maselli, V., Van Damme, R., Fulgione, D. 2013.  
340 Physiological change in an insular lizard population confirms the reversed island  
341 syndrome. *Biol. J. Linn. Soc.* **108**: 144–150.

342 Nevo, E. (1978): Genetic variation in natural populations: Patterns and theory. *Theor.*  
343 *Popul. Biol.* **13**: 121–177.

344 Nietberding, C., Morand, S., Libois, R. and Michaux, J.R. (2006): Parasites and the  
345 island syndrome: the colonization of the western Mediterranean islands by  
346 *Heligmosomoides polygyrus* (Dujardin, 1845). *J of Biogeogr.* **33**: 1212-1222.

347 Olesen, J.M., Valido, A. (2003): Lizards as pollinators and seed dispers- ers: An island  
348 phenomenon. *Trends Ecol. Evol.* **18**: 177–181.

349 Oppliger, A., Clobert, J., Lecomte, J. and Boudjemadi, K. (1998): Environmental stress  
350 increases the prevalence and intensity of blood parasite infection in the  
351 common lizard *Lacerta vivipara*. *Ecol. Lett.* **1**: 129–138.

352 Oppliger, A., Giorgi, M.S., Conelli, A., Nembrini, M., John-Alder, H.B. (2004): Effect of  
353 testosterone on immunocompetence, parasite load, and metabolism in the  
354 common wall lizard (*Podarcis muralis*). *Can. J. Zool.* **82**: 1713–1719.

355 Pereira, L.K.J., Galdino, C.A.B, Nascimento, L.B. (2014): *Tropidophorus torquatus*  
356 melanism. *Herp. Rev.* **45**: 334.

357 Pérez I de Lanuza, G., Font, E. (2015): Differences in conspicuousness between  
358 alternative color morphs in a polychromatic lizard. *Behav. Ecol.* **26**: 1432–1446.

359 Pérez-Mellado, V. (1984): Sobre un ejemplar melánico de *Podarcis hispanica*  
360 (Steindachner 1870). *Doñana, Acta Vert.* **11**: 320–321.

361 Pérez-Mellado, V., C. Corti, Lo Cascio, P. (1997): Tail autotomy and extinction in  
362 Mediterranean lizards: A preliminary study of continental and insular  
363 populations. *J. Zool.* **243**:553–541.

364 Poulin, R. (2006): Variation in infection parameters among populations within  
365 parasite species: Intrinsic properties versus local factors. *Int. J. Parasitol.* **36**:  
366 877–885.

367 Raia, P., Guarino, F.M., Turano, M., Polese, G., Rippa, D., Carotenuto, F., Monti, D.M.,  
368 Cardi, M., Fulgione, D. (2010): The blue lizard spandrel and the island syndrome.  
369 *BMC Evol. Biol.* **10**: 289.

370 Rosenblum, E.B. 2006. Convergent evolution and divergent selection: lizards at the  
371 White Sands Ecotone. *Am. Nat.* **167**: 1–15.

372 Roulin, A., Ducrest, A.L. (2011): Association between melanism, physiology and  
373 behaviour: a role for the melanocortin system. *Eur. J. Pharmacol.* **660**: 226–33.

374 Roulin, A., Jungi, T.W., Pfister, H., Dijkstra, C., 2000. Female barn owls (*Tyto alba*)  
375 advertise good genes. *Proc. R. Soc. Lond.* **267**: 937–941.

376 Roulin, A., Riols, C., Dijkstra, C., Ducrest, A.L. (2001): Female plumage spottiness and  
377 parasite resistance in the barn owl (*Tyto alba*). *Behav. Ecol.* **12**: 103–110.

378 Runemark, A., Hansson, B., Pafilis, P., Valakos, E.D., Svensson, E.I. (2010): Island  
379 biology and morphological divergence of the Skyros wall lizard *Podarcis*  
380 *gaiageae*: A combined role for local selection and genetic drift on color morph  
381 frequency divergence? *BMC Evol. Biol.* **10**:269.

382 San-Jose, L.M., Gonzalez-Jimena, V. (2008): Frequency and Phenotypic Differences of  
383 Melanistic and Normally Colored Common Lizards , *Lacerta (Zootoca) vivipara*  
384 of the Southern Pyrenees (Spain). *Herpetol. Rev.* **39**: 422–425.

385 Santiago-Alarcon, D., Whiteman, N.K., Parker, P.G., Ricklefs, R.E., Valkiu, G. (2008):  
386 Patterns of parasite abundance and distribution in island populations of  
387 Galápagos endemic birds. *J. Parasitol.* **94**: 584–590.

388 Trocsanyi, B., Korsos, Z. (2004): Recurring melanism in a population of the common  
389 wall lizard: numbers and phenotypes. *Salamandra* **40**: 81–90.

390 Trapanese, M., Buglione M., Maselli V., Petrelli S., Aceto S., Fulgione, D. (2017): The  
391 first transcriptome of Italian wall lizard, a new tool to infer about the Island  
392 Syndrome. *PlosOne* 12:e0185227.

393 True, J.R. (2003): Insect melanism: the molecules matter. *Trends Ecol. Evol.* **18**: 640–  
394 647.

395 Turner, J.R.G. (1977): Butterfly mimicry: the genetical evolution of an adaptation.  
396 *Evol. Biol.* **10**: 163–206.

397 Vervust, B., Grbac, I., Van Damme, R. (2007): Differences in morphology,  
398 performance and behaviour between recently diverged populations of *Podarcis*  
399 *sicula* mirror differences in predation pressure. *Oikos* **116**: 1343–1352.

400 Wiernasz, D.C., (1989): Female choice and sexual selection of male wing melanin  
401 pattern in *Pieris occidentalis* (Lepidoptera). *Evolution* **43**: 1672–1682.

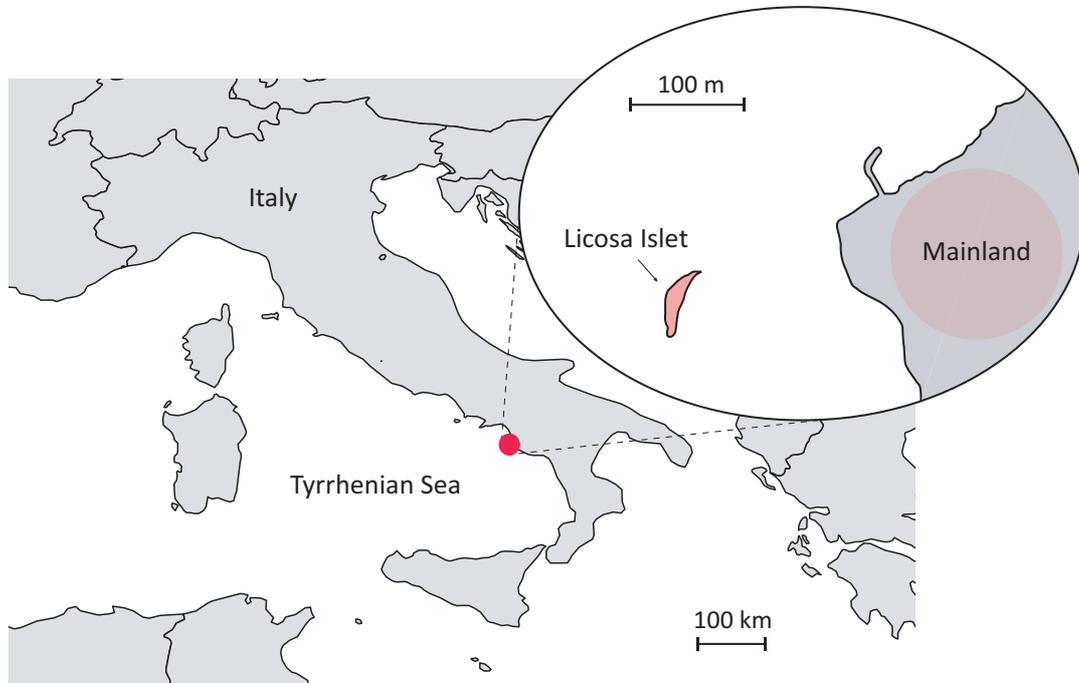
402 Wilson, K., Lotter, S.C., Reeson, A.F., Pell, J.K. (2001): Melanism and disease  
403 resistance in insects. *Ecol. Lett.* **4**: 637–649.

404 Zuffi, M. (1986): Su *Podarcis muralis maculiventris* (Werner, 1891) melanica in risaia  
405 a Bereguardo (Pavia) (Reptilia Lacertidae). *Atti. Soc. Ital. Sci. Nat. Mus. Civ. Stor.*  
406 *Nat. Milano* **127**: 293–296.

407

408

409 **Fig. 1** — Geographic location of the two populations of study: the melanistic  
410 population of *Podarcis siculus* (Licosa Islet), and the ‘normally’-coloured population  
411 (mainland). Red indicates sampling area.



412

413

414

415

416

417

418

419

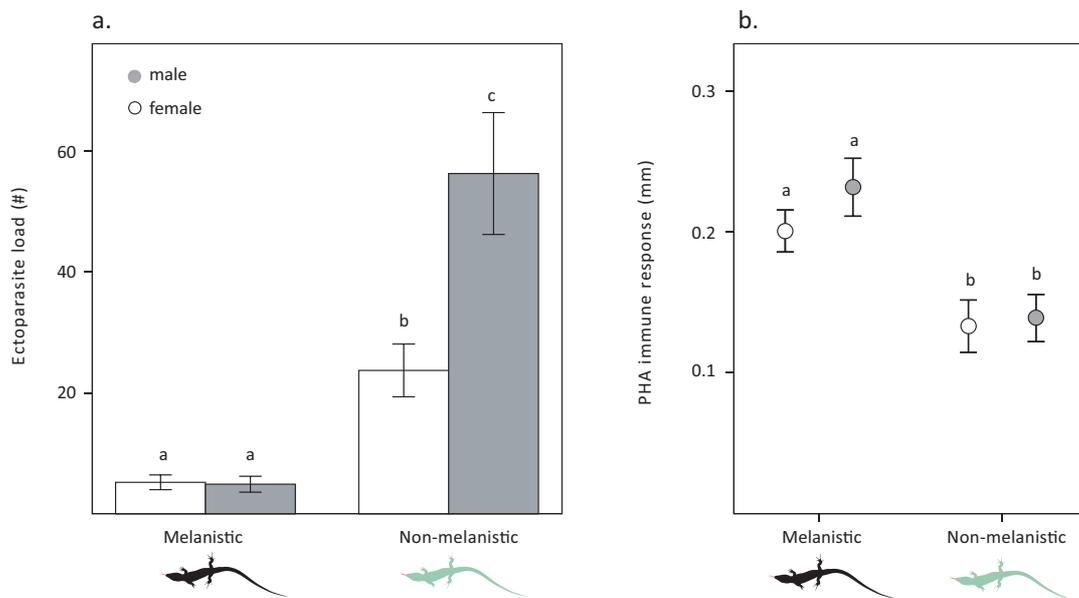
420

421

422

423

424 **Fig. 2** — Mean (a) ectoparasite infestation and (b) cellular immune response to PHA  
425 injection in males (grey) and females (white) lizards from a melanistic population and  
426 ‘normally’-coloured population of *Podarcis siculus*. Error bars represent SE. The same  
427 letters above the bars denote that means are not significantly different from each  
428 other.



429